DNA QIACUBE DIFFERENTIAL EXTRACTIONS

A. SCOPE

This protocol employs the QIAamp DNA Mini Kit, a Digest Buffer, dithiothreitol (DTT), and the QIAcube for the automated separation and DNA extraction of epithelial and sperm cell fractions. The QIAcube controls integrated components including a centrifuge, heated shaker, pipetting system, and robotic gripper.

B. QUALITY CONTROL

B.1 Protective gloves, a lab coat, eye protection and a mask must be worn at all times when performing this procedure.

B.2 Each new QIAamp DNA Mini Kit lot must undergo quality control testing prior to extracting casework samples.

B.3 Biological material with known results along with a reagent control will be extracted using all the components of the kit undergoing quality control testing. The extracted material will be carried though the entire DNA analysis process. The results obtained from the known extracted sample must be as expected and good quality, as described in the GlobalFiler interpretation guidelines (DOC ID 12628), for the kit to pass quality control testing. The quality control data will be stored in our Laboratory Asset Management System (LAM).

B.4 An analyst that dilutes the concentrated Buffers AW1 and AW2 prior to their initial use will be watched by a second individual from the Biology Unit to confirm correct preparation; this second individual can be another analyst, an Investigative Assistant, etc. Both individuals will initial the bottle. In addition, the lot number and expiration date of the added ethanol will be recorded on the bottle.

B.5 See (DOC ID 1835) to determine reagent expiration dates.

B.6 Digest Buffer and DTT will undergo quality control testing prior to being used in the differential extraction of casework samples.

B.7 Samples known to contain a mixture of sperm and epithelial cells with known results along with a reagent control will be extracted using the Digest Buffer / DTT undergoing quality control testing. The extracted sperm and epithelial fractions will be carried through the entire DNA analysis process. The Digest Buffer / DTT will pass quality control testing when a good quality DNA profile, as described in the GlobalFiler as interpretation guidelines (DOC ID 12628), is obtained with the expected results for each fraction. The quality control data will be will be stored in our Laboratory Asset Management System (LAM). If the same lot number of powder DTT as previously quality control tested needs to be prepared, the analyst preparing the new batch of DTT will be watched by a second analyst to confirm correct preparation. Both analysts will initial the box. No additional quality control testing is necessary.

B.8 At least two reagent controls must be extracted along with a set of questioned samples.

B.9 Do not use spray bottles to spray cleaner onto surfaces of the QIAcube workstation.

B.10 Do not use alcohol or alcohol-based solutions to clean the QIAcube door. Clean the QIAcube door with deionized water.

B.11 Do not submerge buffer bottles in 70% alcohol as the blue ring is not ethanol resistant.

B.12 To clean touch screen, moisten a kimwipe with water and carefully wipe display. Wipe dry with a kimwipe.

B.13 Do not use bleach, solvents, or any reagents containing acids, alkalis, or abrasives to clean the QIAcube and its accessories; instead use Decon-Quat 200 C.

B.14 Empty the waste drawer and decontaminate the QIAcube with Decon-Quat 200 C after each use.

B.15 When consuming a sample and the corresponding extract, you must keep the post extraction substrate (refer to the DNA Quality Manual DOC ID 1833 for details on evidence consumption and retention).

C. SAFETY

C.1 Protective gloves, scrubs, a lab coat, and a mask must be worn at all times when performing this procedure. Additionally, eye protection (e.g. safety glasses or a face shield) must be worn if this procedure is performed outside of a hood.

C.2 The sample preparation waste contains guanidine hydrochloride from Buffers AL and AW1, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with water or ethanol.

C.3 All appropriate SDS sheets must be read prior to performing this procedure.

C.4 Treat all biological specimens as potentially infectious.

C.5 Distinguish all waste as general, biohazard, or sharps and discard appropriately.

D. REAGENTS, STANDARDS AND CONTROLS

D.1 QIAamp DNA Mini Kit

D.1.1 Buffer AL

D.1.2 Proteinase K

D.1.3 Buffer AW1

Before using for the first time, add 125 mL ethanol (Absolute) to 95 mL AW1 concentrate.

D.1.4 Buffer AW2

Before using for the first time, add 160 mL ethanol (Absolute) to 66 mL AW2 concentrate.

D.1.5 Buffer AE

D.2 Absolute Ethanol (200 proof)

D.3 DTT, 1M Dithiothreitol, 5 mL

Dissolve 0.77 g dithiothreitol in 5 mL sterile deionized water in a sterile, disposable plastic 15 mL tube or original container, or add 6493 µL of deionized water to the bottle containing 1 g of the dithiothreitol. Store 110 µl aliquots in 0.5 mL microcentrifuge tubes at approximately -20° C. Discard any unused portion of a thawed tube.

D.4 Digest Buffer

10Mm Tris-HCl - 10mM EDTA - 50mM NaCl - 2% SDS, pH 7.5, 500 mL

Add 5mL 1 M Tris-HCl (pH 8.0), 10 mL 0.5 M EDTA (pH 8.0), 5 mL 5 M NaCl, and 100 mL 10% SDS to 380 mL deionized water (or use 0.29 g NaCl, e.g. Sigma S-3014 molecular biology grade, in place of the 5 mL 5 M NaCl and adjust volume to 500 mL). Store at room temperature.

D.5 Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner (Decontamination)

D.6 Decon-Quat 200 C (Decontamination of QIAcube)

E. EQUIPMENT & SUPPLIES

E.1 Equipment

E.1.1 QIAcube

E.1.2 Scissors/Forceps

E.1.3 Microcentrifuge

E.1.4 Eppendorf ThermoMixer

E.1.5 Eppendorf Smartblock 1.5 mL

E.1.6 Eppendorf Smartblock 2.0 mL

E.1.7 Eppendorf ThermoTop

E.1.8 Pipettes

E.1.9 Vortexer

E.2 Supplies

E.2.1 Kimwipes

E.2.2 Microcentrifuge tubes

E.2.3 Spin baskets

E.2.4 Sterile aerosol resistant pipette tips

E.2.5 Microcentrifuge tube racks

E.2.6 Permanent marker

E.2.7 QIAcube sample tubes (2 mL, Qiagen P/N 990381)

E.2.8 QIAcube Filter Tips, sterile aerosol-resistant

E.2.9 Shaker racks

E.2.10 QIAamp mini rotor adapters (Part of Qiagen kit)

E.2.11 Spin columns (Part of Qiagen kit)

E.2.12 QIAcube elution tubes (Part of Qiagen kit)

E.2.13 Rotor adapter tray (Part of Qiagen kit)

E.2.14 Disposable gloves

E.2.15 Mask

E.2.16 Lab coat

E.2.17 Eye protection (e.g. safety glasses, face shield)

E.2.18 Decon-Quat 200C

E.2.19 Extraction sheet

F. PROCEDURES

Note: *The QIAcube can process a maximum of twelve samples at a time per machine. It cannot process one or eleven samples. This protocol is to be used only on QIAcubes #3 (S/N 7918) and #4 (S/N 7920).*

F.1 Cut the sample and place into a labeled 2 ml QIAcube sample tube. Add 500 µL Digest Buffer and 15 µL Proteinase K.

F.2 Vortex for 10 seconds. Incubate at approximately 56°C with approximately 550 rpm mixing for at least 1 hour and no more than 2 hours using a ThermoMixer. Lyses time will vary depending on the size and density of the source material. This incubation time must be recorded and can be documented as a start and end time or total incubation time.

F.3 Briefly centrifuge the sample to remove drops from inside the lid. Using a pipette tip or sterile forceps remove substrate and place in a spin basket. Place basket back into collection tube and centrifuge for 5 minutes at max speed to collect any fluid remaining in substrate.

F.4 Remove the spin basket from tube (The substrate may be retained and stored, if required). Vortex the tube on high speed for 10 seconds. Briefly centrifuge.

F.5 Transfer lysate to the labeled 1.5mL Elution Tubes and place in the Rotor Adapter.

F.6 Load two trays of dark grey tips (1000µL wide-bore) onto the QIAcube deck. **Make sure that the tips contain no pieces of plastic that may have chipped off of the plastic holder during shipment.**

F.7 Ensure the reagent bottle rack is in place on the worktable. Add Digest Buffer to a sterile 30mL reagent bottle and place in position 1 of the reagent bottle rack.

OPTIONAL: Add deionized water to a sterile 30mL reagent bottle and place in position 2 of the reagent bottle rack.

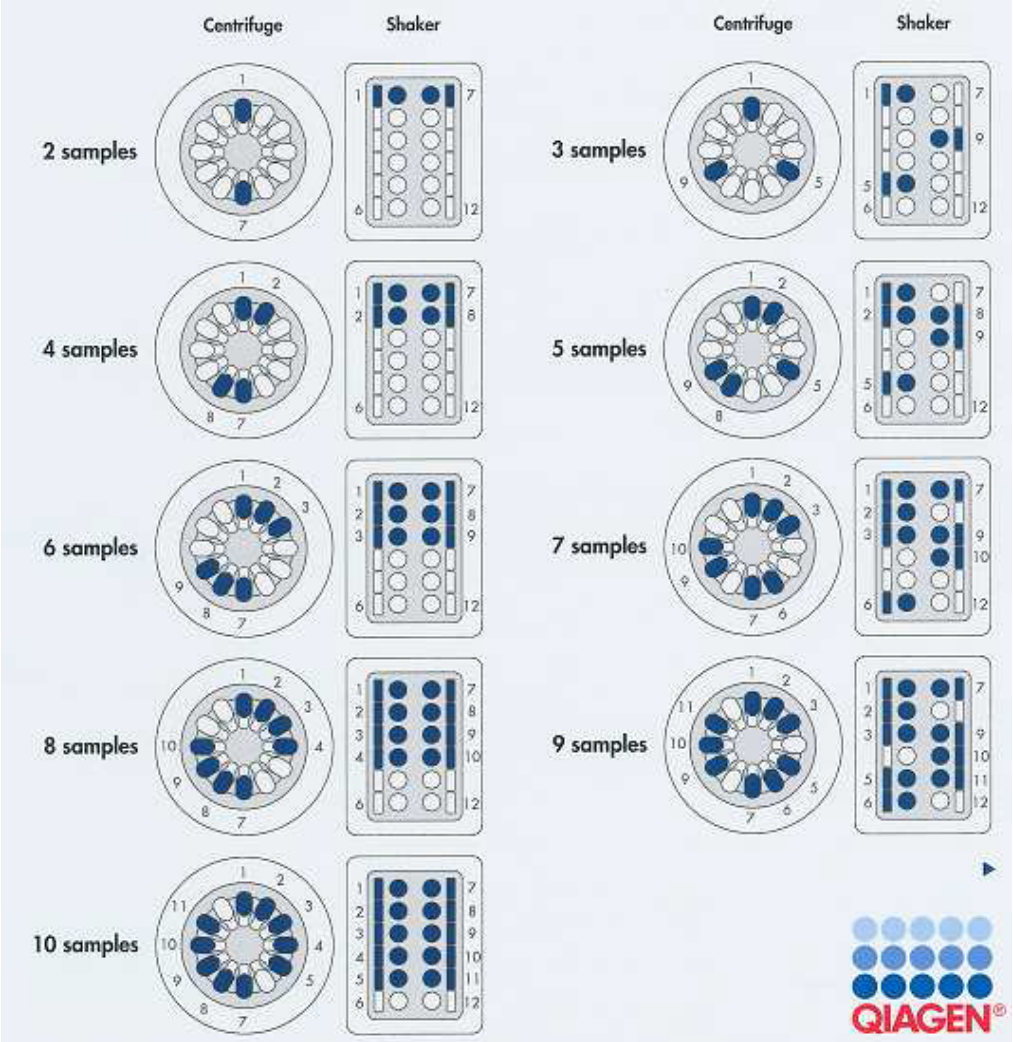
F.8 Prepare the Sperm Digest Buffer for the appropriate number of samples as indicated by the table below. Add buffer to a sterile elution tube and place in microcentrifuge tube slot A. Unlike sperm wash buffer, Sperm Digest Buffer must be prepared fresh for every run and cannot be re-used or "topped-off."

NOTE: For the modified water wash protocol, the Sperm Digest Buffer will be added manually, therefore, include one extra sample when making Sperm Digest Buffer to account for loss during pipetting.

Sample Number	QIAcube Protocol	Total Sperm Digest Buffer Volume	Digest Buffer	Proteinase K	1 M DTT
2	S&L 12B	352.3	268.4	16.8	67.1
	S&L 6	529.5	403.4	25.2	100.9
3	S&L 12B	507.6	386.7	24.2	96.7
	S&L 6	764.4	582.4	36.4	145.6
4	S&L 12B	663.9	505.8	31.6	126.5
	S&L 6	999.3	761.3	47.6	190.4
5	S&L 12B	819.1	624.1	39	156
	S&L 6	1234.2	940.3	58.8	235.1
6	S&L 12B	975.4	743.1	46.5	185.8
	S&L 6	1469.1	1119.3	70	279.8
7	S&L 12B	1131.7	862.2	53.9	215.6
8	S&L 12B	1287	980.5	61.3	245.2
9	S&L 12B	1443.1	1099.5	68.7	274.9
10	S&L 12B	1598.4	1217.8	76.1	304.5
12	S&L 12B	1910.9	1455.9	91	364

F.9 Place sterile, labeled 2mL QIAcube tubes into appropriate positions of the shaker table. Ensure all 2.0 mL tube caps are locked into position. Epithelial factions will be dispensed into these tubes and sperm fractions will remain in elution tubes on the rotor adapters.

F.10 In Step F.5, digested samples were placed into rotor adapters. Now place the rotor adapters into appropriate centrifuge buckets.



F.11 Close the lid of the QIAcube and turn the machine on (if it is not already on). To select the separation and lysis protocol press "DNA" to select DNA protocols. Using the up or down arrows, highlight "Pipetting" and press "Select". Select "Epithelial and Sperm Cell". If 2-6 samples are being extracted, highlight and select "Separation and Lysis 6." If 7-12 samples are being extracted, two separate protocols are needed- "Separation and Lysis 12 A" and "Separation and Lysis 12 B." If performing the modified water wash protocol sperm cell staining, two separate protocols are needed- "Separate and Lysis 12A Mod" and "Separate and Lysis 12B Mod". Select the required protocol, press "Start**",** and follow the prompts the QIAcube will give. After the user has selected "yes" to all questions, the robot will begin the separation and lysis protocol.

F.12 After the completion of "Separation and Lysis 12 A" or "Separate and Lysis 12A Mod" the QIAcube will stop. Empty the waste drawer containing the used tips into approved biohazard waste container and refill tip rack with two cartridges of 1000µL wide-bore filter tips**. OPTIONAL**: The epithelial fraction tubes may be removed from the shaker rack at this point and proceed to Step F.17.

F.13 "Separation and Lysis 12 B" or "Separate and Lysis 12B Mod" can now be started. It may be necessary to add more deionized water and/or Digest Buffer to their respective reagent bottles; the software will prompt you during the load check if this is necessary.

F.14 After the completion of the separation and lysis protocol empty the waste drawer containing the used tips and dispose of the remaining sperm lysis buffer. The remaining Digest Buffer can be re-used. Remove rotor adapters from centrifuge buckets and epithelial fraction tubes from the shaker rack (if not already done so) and proceed to sperm lysis.

F.15 **OPTIONAL:** Re-suspend the pellet in the remaining 50 µL of water. Remove 3 µL (or approximately 10% of the sample) of re-suspended cells and place on a slide. Perform Christmas Tree staining and examine microscopically for the presence of spermatozoa (Document 1600).

F.16 **OPTIONAL:** Add 160µl of Sperm Digest Buffer, created in step F.8, to each sample and vortex.

F.17 Place sperm fraction tubes in a Thermomixer pre-heated to 70°C.Incubate for 10 minutes at 900 rpm. Remove the samples and vortex vigorously for 10 seconds. Centrifuge briefly.

F.18 Add 500µL of AL Buffer to BOTH sperm and epithelial fractions and incubate for 10 minutes at 56°C in the Thermomixer.

F.19 Transfer the lysed sperm faction (after heating) from elution tubes to labeled 2mL QIAcube tubes.

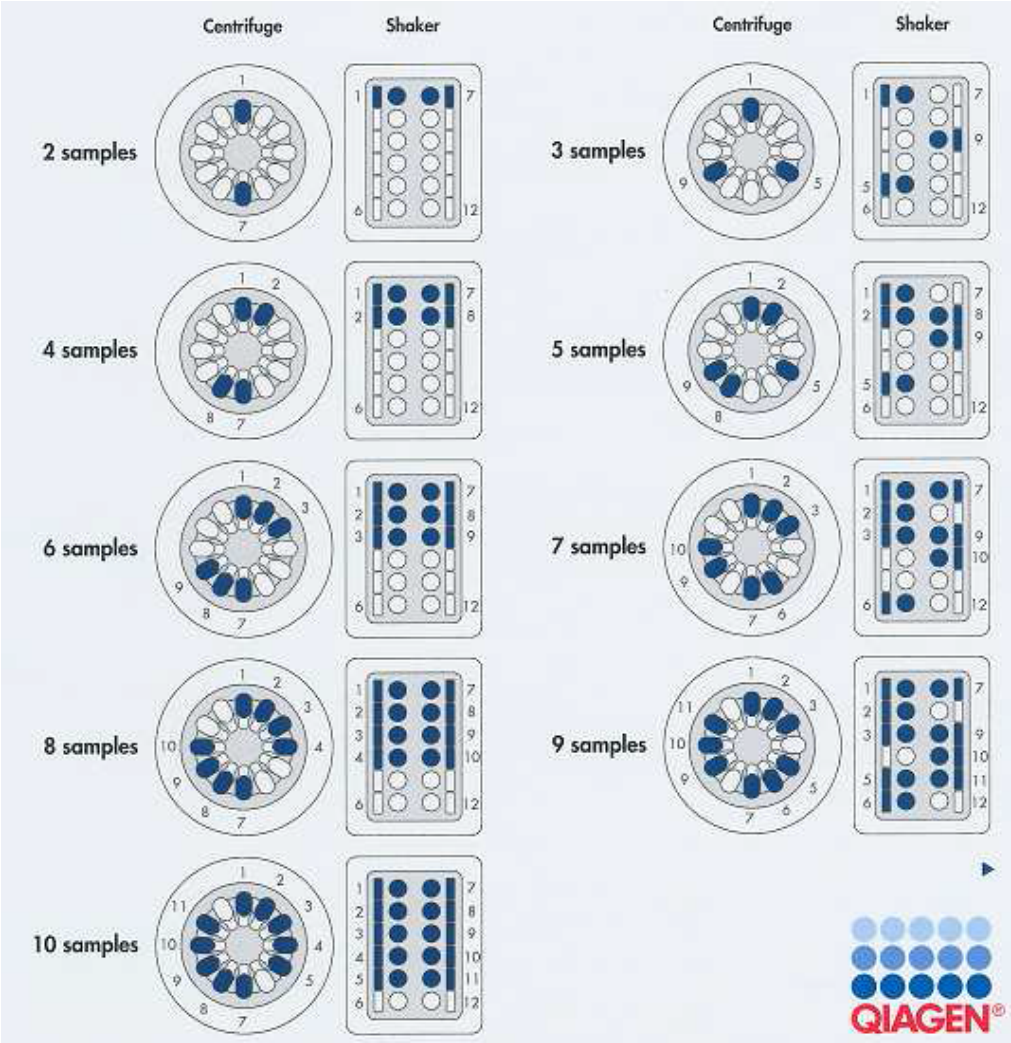
F.20 Both Sperm and Epithelial fractions are now ready for purification on the QIAcube.

F.21 Load a tray of light grey tips (1000µL) and a tray of blue tips (200µL). Fill the reagent bottles with their respective reagents to just under the fill line.

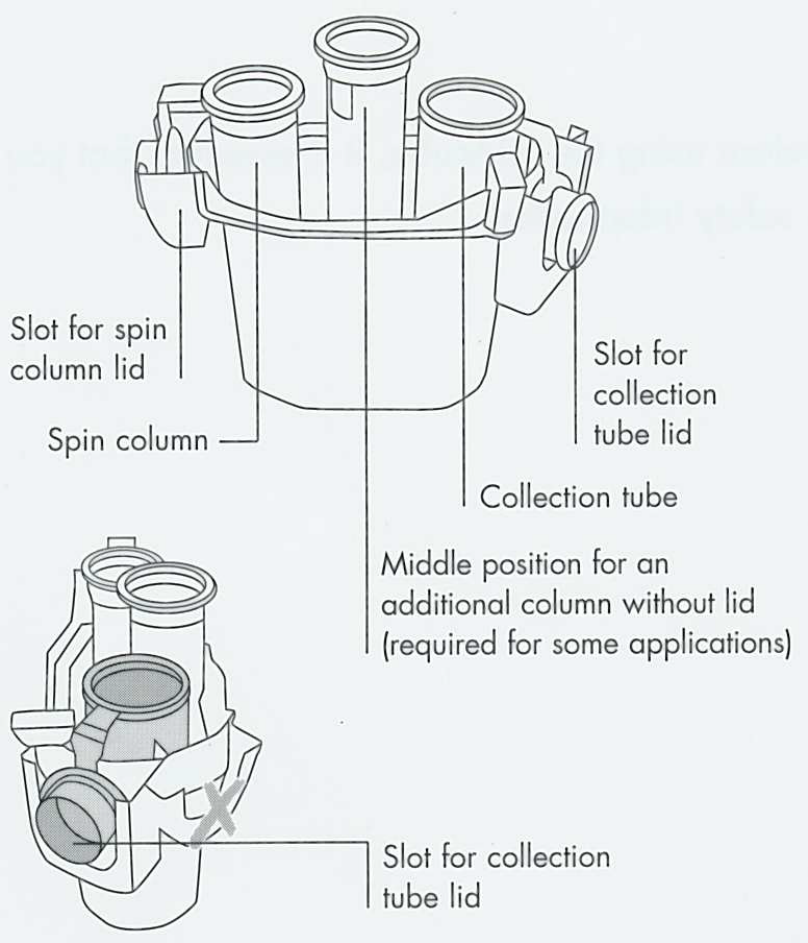
F.22 Add the reagent bottles to their respective positions in the reagent bottle rack as seen in the diagram below.

EtOH	AW1
AW2	AE

F.23 Load samples into the shaker rack according to the diagram below. Ensure that all 2.0mL tube caps are locked into position.



F.24 Assemble rotor adapters with spin column and properly labeled 1.5mL collection tubes (These will contain the final sample). Refer to the diagram below to assemble one rotor adapter per sample.



F.25 Load the centrifuge with assembled rotor adapters.

F.26 Close the lid of the QIAcube. To select the elution protocol, select "DNA", "QIAamp DNA Blood Mini", "Buccal swab lysate", and "Edit Elution". Alternatively, select the lysate protocol shortcut displayed on the touch screen. Press start and follow the prompts seen on the QIAcube touchscreen. After the user has selected "yes" to all prompts the robot will begin the elution protocol.

Note: The elution volume can be adjusted to elute in 50-100 uL. To adjust the elution volume press "Edit", then using the arrows, highlight the "elution volume", and then press "Select". Change the elution volume by pressing "+" or "-".

F.27 After the elution protocol is completed, remove the rotor adapters from the centrifuge. Remove and discard the QIAamp spin column from the 1.5 mL tubes. Cap and remove the 1.5 mL tubes containing the extracted material. Discard the rotor adapters.

F.28 Cap reagent bottles. Empty and clean the tip waste drawer. Wipe down the deck of the QIAcube and deck items with Decon-Quat 200 C. Clean the shaker rack with Decon-Quat 200 C followed by a rinse with deionized water; bleach based solutions must not be used on the shaker rack.

F.29 Save all data files associated with a QIAcube extraction on the USB stick provided with the QIAcube. These files should then be transferred from the USB stick to each analyst's casework folder on the "I" drive. ONLY use the supplied QIAcube USB stick on the QIAcube to save all associated data files. To save the data files do the following: in the main menu press "Tools", "Data exchange", "Select", "Save all files", then "OK". These files must be saved on the same day of use to prevent loss of data; the Qiacubes can only retain nine report files at a time.

F.30 Quantitate (DOC ID's 1784 and 1785) the DNA and concentrate samples (DOC ID 1780) as necessary. Alternatively, samples may be concentrated prior to quantitation. Store sample extracts in the refrigerator when not in use. Sample extracts may be frozen for long-term storage.

G. INTERPRETATION GUIDELINES

Not applicable

H. REFERENCES

H.1 QIAcube User Manual, Qiagen, 6/2008

H.2 EZ1® Differential Extraction on the QIAcube, Marshall University, 01/2014